Exhibit A

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Composition for treating wounds which comprises fibroblasts harboring a foreign gene

The present invention relates to a composition for treating skin damage which can only be treated with difficulty. In the case of burns covering a large area, it can represent a substantial problem to restore the regions of the skin which have been destroyed by the damage, in particular when major areas of the skin have been severely damaged. It is frequently also quite a substantial problem to restore the damaged skin regions in the case of other diseases such as neoplastic diseases or chronic skin lesions.

Gene-transfected fibroblasts per se are already known from the state of the art. International Patent Application WO 95/07105 describes a method for inhibiting or preventing the growth of tumor cells in the central nervous system of a patient in which the immune response of the patient is stimulated by immunization with either gene-transfected tumor cells or unmodified tumor cells and gene-transfected, autologous fibroblasts. The cytokines which are used are first and foremost those which stimulate the immune system.

DE-OS 44 06 073, which was also filed in the name of the Albert Ludwigs University Hospital complex, Freiburg, relates to a general method for preparing human, clonogenic fibroblasts and to a method for gene-transfecting fibroblasts. This application does not disclose topical application or gene transfection specifically with genes which promote wound healing.

WO 92/15676 describes the use of gene-transfected fibroblasts for somatic gene therapy. In this case, the transfected fibroblasts are fixed in an extracellular collagen matrix and implanted **under** the skin. The object of this application is aimed at

remedying genetic defects in the human genome. In this context, a functionally active "replacement" gene is introduced into a somatic cell and used to compensate for the damage arising as a result of the defective gene. This literature reference does not address the treatment of wounds or cytokines which promote wound healing, such as TGF-α, EGF or b-FGF. This citation mentions, in particular, cytokines which possess other biological properties, such as GM-CSF, TNF or EPO. The fibroblast growth factor which is mentioned in claim 5 is used as a blood vessel-forming substance.

Various growth factors which have an effect on keratinocytes are also known. WO 90/08771 relates to the recombinant preparation of growth factor proteins which affect epithelial cells. However, the human keratinocyte growth factor is used as an essentially pure protein in this application.

US Patent Specification 5,302,701 describes the development of an artificial polypeptide which is cell-adhesive and also possesses cell growth-promoting activity. The polypeptide in this case is a combination of fibronectin and fibroblast growth factor (FGF).

While US Patent 5,196,196 relates to a wound dressing which also comprises a support matrix, there is an important difference from the present invention with regard to the principle by which the method works and also the active agent. The US patent uses purified protease nexin I (PN-I) in the wound dressing. However, this protease is not a growth factor but an enzyme having defined properties. Protease nexin I is a serine protease inhibitor; i.e. a member of the serine protease superfamily which is synthesized and secreted by human fibroblasts in culture. The US patent uses the protein in purified form and not in the form of a gene which is expressed by fibroblasts into which this gene has been incorporated.

Furthermore, the use of cultured, autologous keratinocytes, which are suspended in fibrin adhesive, for treating extensive burn wounds is known from the

state of the art [Stark et al., Eur. J. Plast. Surg. (1995) **18**, pp. 267-271]. An attempt has also already been made to use keratinocytes which have been transfected in situ by means of particle DNA transfer in wound healing in a pig model [Andree et al., Proc. Natl. Acad. Sci. USA, (1994) **91**, pp. 12188-12192].

Andreatta-van Leyen et al. (J. Biomedical Materials Res., Vol. 27 (1993), pp. 1201-1208] describe a wound bandage which comprises gene-transfected keratinocytes which produce bovine growth hormone (bGH).

Some of the proposed solutions known from the state of the art rely partly upon keratinocyte preparations (e.g. so-called keratinocyte sheets) which are very difficult to handle and can often only be used when three-dimensional structures have formed during the cell culture. There is the risk that allogenic preparations, in particular those which come from different donors and have not been characterized, are contaminated with viruses (HIV, HCV or viruses which have not yet been characterized) and that the patient who is to be treated can be infected by these viruses.

The present invention therefore relates to compositions for treating wounds, which compositions comprise fibroblasts, which harbor at least one foreign gene encoding a wound healing-promoting cytokine, and at least one additional wound healing-promoting component. In addition to this, the composition according to the invention can comprise further components which are customarily used in such compositions.

The additional wound healing-promoting component can be a so-called fibrin adhesive. Such fibrin adhesives are commercially available and are used in various areas of medicine, in particular in surgery.

In principle, fibrin adhesion makes use of the last phase of blood coagulation. The fibrin adhesive contains fibrinogen, which is converted by thrombin into monomeric fibrin. This in turn forms aggregated fibrin by means of end-to-end or side-to-side addition.

Furthermore, in a preferred embodiment, the fibrin adhesive contains fibronectin. At the same time, thrombin activates factor XIII, which is normally present in adequate quantity in the fibrin adhesive. As a further component, the fibrin adhesive contains a thrombin solution which is frequently added, together with calcium chloride, as a separate component.

In addition to this, the fibrin adhesive can also contain sufficient quantities of albumin or plasminogen.

In a preferred embodiment, the additional wound healing-promoting component can also be a solid constituent which serves as the support matrix. Supports of this nature for "artificial skin" are commercially available (for example Laserskin® or Biobrane®). The solid components can preferably be a matrix composed of a derivative of hyaluronic acid, preferably a hyaluronic acid ester. Hyaluronic acid is an endogenous constituent of the connective tissue which is subject to an extraordinary high turnover rate. Therefore, when the matrix is composed of hyaluronic acid, it is very rapidly degraded in the wound by the endogenous hyaluronidase. For this reason, preference is given, according to the invention, to using those derivatives of hyaluronic acid which are degraded somewhat more slowly in the body.

A particular advantage which can be achieved by using a support matrix is that cells which have still not formed a confluent association of cells can be applied to the wound. When keratinocytes are also employed in the composition according to the invention, these cells can, with the help of the support matrix, be used in a subconfluent state. At this time, they are in their phase of optimal growth and are still dividing very frequently and react particularly well to the cytokines which are produced by the gene-transfected fibroblasts.

In a preferred embodiment, the composition according to the invention can also comprise

keratinocytes, preferably autologous keratinocytes. The keratinocytes are particularly involved in forming the outer layer of the skin, the so-called epidermis. Keratinocyte cultures can be cultured using a routine technique which is known per se [e.g. Rheinwald et al. Nature 265 (1977) pp. 421-424]. In this technique, a small piece of skin is normally removed by biopsy and the epidermis and dermis are then separated from each other. A cell suspension is prepared from the epidermis, preferably by treating the latter with a proteolytic enzyme. The individual cells which result are then propagated under sterile conditions in culture vessels (flasks or dishes) and detached from the culture vessels at a suitable point in time.

The composition according to the invention comprises genetically altered fibroblasts. Fibroblasts are mesenchyme-derived cells which have a large cell body and a somewhat flattened nucleus. The fibroblasts are particularly involved in forming the intercellular substance of the connective tissue. According to the invention, either autologous fibroblasts can be used or else, in a preferred form, use is made of allogenic fibroblasts which are obtained from another individual, that is not from the patient to be treated. Very particularly preferably, use is made of fibroblasts which have been clonally selected, i.e. which derive from one clone.

In a particularly preferred embodiment, the gene-transfected fibroblasts have been treated with a dose of ionizing radiation such that they are no longer able to replicate and die after a given period of time. An advantage of this irradiation is that the gene-transfected cells die in the body after a relatively short and clear-cut period of time (< 3 weeks). An additional advantage of using irradiated fibroblasts is that the cells which have been treated in this way still continue to express the cytokine satisfactorily.

In a particularly preferred embodiment, use is made, according to the invention, of cells from

immortalized fibroblast cell lines, in association with which a cell line which has been given the designation KMST-6 has proved to be particularly satisfactory. Immortalized fibroblast cell lines are advantageous since they can be cultured continuously and can be used in a precisely characterized manner from the biological point of view. However, it is also possible to use other suitable immortalized fibroblast cell lines without any difficulty.

A foreign gene which encodes a suitable cytokine is introduced into the fibroblasts which are used in accordance with the invention. This foreign gene is preferably a gene which encodes a cytokine such as EGF, TGF-α or KGF.

The epidermal growth factor is termed EGF for short. This factor is a globular protein of about 6.2 kDa which possesses 53 amino acids. According to the invention, it is not absolutely necessary for the complete gene to be introduced into the transfected fibroblasts; it is sufficient for that part which possesses the biological activity to be inserted. In order to enable the biologically active peptide to be secreted, the appropriate cDNA moiety is preferably fused in-frame to the secretory signal sequence of human G-CSF. According to the invention, preference is also given to using EGF-like proteins, such as transforming growth factor α (TGF-α), which exhibits a high degree of homology with EGF. The biological activities of EGF and TGF-α are comparable. Both cytokines exert an effect on epidermal development and cell differentiation.

NGF (nerve growth factor) may also be used in a preferred manner. This cytokine is in particular responsible for the survival, differentiation and functional activity of sensory and sympathetic neurons in the peripheral nervous system. The accelerating effect on wound healing is probably due to the ability of NGF to increase the survival and functional activities of a variety of immunocompetent cells such

as granulocytes, mast cells, macrophages and lymphocytes.

Another cytokine which is preferably employed is keratinocyte growth factor (KGF), which in particular stimulates the division of keratinocytes which are able to divide. According to the invention, variants of the genes can also be employed. Such variants can exhibit deletions or additions and the sequence can be altered in a controlled manner. It is also perfectly possible to employ cytokine variants which have a higher biological activity than do the naturally occurring cytokines, such as, in particular, constructs obtained by fusing two or more cytokines.

The fibroblasts which are employed in accordance with the invention harbor a foreign gene which encodes a cytokine which promotes wound healing. While this foreign gene can preferably be derived from humans, it can also be derived from an animal such as a mouse or a cow. However, a prerequisite is that the cytokine is not species-specific when the foreign gene comes from another species.

The foreign gene has to be introduced into the fibroblasts. This can be brought about by the desired gene being incorporated into a suitable vector and then being transfected into the fibroblasts. Suitable vectors are viral vectors or plasmid vectors, with particular preference being given to plasmid vectors since, when viral vectors are used, additional investigations have to be carried out in order to exclude the possibility of contamination with replication-competent viruses.

The compositions according to the invention exhibit a variety of advantages as compared with the solutions known from the state of the art.

The genetically altered fibroblasts which, in a preferred embodiment, have been irradiated lethally, can express the desired cytokine(s) for a predetermined time and secrete it(them) into the tissue. Because the cytokine is being produced continuously, it is possible

to circumvent the problems which are due to the short half-life of cytokines when they are applied externally in a local manner. In the compositions according to the invention, different cell types (keratinocytes/fibroblasts) can be combined with each other with it also being possible for the gene-transfected fibroblasts to contain different cytokines. This makes it possible to introduce different growth factors into the wound region in a deliberate manner. If autologous keratinocytes are used, it is then possible to avoid the histoincompatibility rejection reaction.

It is also conceivable to additionally introduce genes for growth factors, such as G-CSF or GM-CSF, which act on white blood corpuscles for the purpose of stimulating the endogenous immune defense in the wound regions. This can boost the body's own defense against infections, something which is particularly relevant in the case of burn wounds.

It is also perfectly possible to use the composition according to the invention without any keratinocytes. In this case, the genetically altered fibroblasts release the cytokine into the environment and the treated patient's keratinocytes which are located, for example, in the marginal regions of the wound are stimulated.

The compositions according to the invention are preferably in the form of pharmaceutically acceptable formulations. These formulations can either be suspensions of fibrin adhesive and gene-transfected fibroblasts which can be applied to the wound as solutions, suspensions or ointments or in the form of gels. When the composition comprises a solid component, that is a support matrix, they are then preferably present in the form of sterile units which can be preserved in a suitable manner. Such preparations can, for example, be in the form of deep-frozen, sterile dressings.

Description of the figures

Figure 1 shows the structure of the vector for expressing human EGF. Part (A) shows the plasmid construct containing the chimeric EGF gene. Part (B) shows the sequence of the in-frame fusion between the human G-CSF signal sequence (underlined) and the mature, human EGF-encoding region (Asn Ser Asp ...).

Figure 2 shows the secretion of EGF by fibroblasts which have been transfected with the plasmid pCMV-EGF-IRES-TKNeo. The fibroblasts are fibroblasts of the KMST-6 cell line which have been irradiated (100 Gy). In the experiment, 2×10^5 irradiated cells were sown on six-well culture plates and the concentration of EGF was determined in the culture supernatants after 24 hours. The values are mean values.

Figure 3 shows the bioactivity of chimeric EGF polypeptides which were obtained from clones #3 and #6 of gene-transfected KMST-6 fibroblasts.

Part (A) shows the bioactivity on BALB/MK strain mouse keratinocytes.

Part (B) shows the results which were obtained using human primary keratinocytes. The values indicate the average results from eight measurements, together with the standard deviation. The dotted bars represent the calibration curve which was obtained using recombinant EGF. The empty bars represent the control values which were obtained using fibroblasts of the KMST-6 cell line. The hatched and gray bars represent the results which were obtained using culture supernatants from the two clones 3 and 6, respectively.

Figure 4 shows the proliferation of primary human keratinocytes in a culture test using irradiated fibroblasts of the KMST-6 cell line which had been gene-transfected.

Part (A) shows the results after four days of coculture.

Part (B) shows the results after 10 days of coculture.

The present invention is clarified further by the examples which are listed below:

Example 1

Constructing a plasmid which contains a gene for EGF, and transfection

In most human cells, EGF is synthesized as a 130 kDa transmembrane glycoprotein precursor molecule which is proteolytically cleaved into the biologically active 6.2 kDa EGF peptide containing 53 amino acids. In accordance with the invention, therefore, a chimeric construct was prepared which encodes an in-frame fusion of the mature EGF peptide and the human G-CSF secretory signal sequence. The structure is depicted diagrammatically in Figure 1 (A). The DNA fusion fragment which resulted was placed under the transcriptional control of the human CMV (cytomegalovirus) promoter and incorporated into a dicistronic vector in order to bind the expression of the transgene to that of the neomycin phosphotransferase selection marker.

In the cloning, the sequence encoding the mature human EGF peptide was amplified by PCR technology and the resulting product was subcloned into the pBluescript (Stratagene) vector and subsequently sequenced. The human G-CSF signal sequence was likewise amplified from human G-CSF cDNA by means of PCR technology, with an improved Kozak consensus sequence at the 5' end and a single NheI restriction cleavage site being created at the same time. The relevant sequence is depicted in Figure 1 (B).

The plasmid which had been produced in this way was transfected into human fibroblasts of the KMST-6 cell line, and all the neomycin-resistant clones secreted human EGF, as was demonstrated by means of an ELISA test. The control showed that untransfected human fibroblasts of the KMST-6 cell line did not secrete any detectable quantities of hEGF either before or after irradiation.

Clones #3 and #6, which secreted 37 and 8 ng of EGF/10^6 cells and 24 hours, respectively, were used for the subsequent investigations.

The secretion of human EGF into the supernatant by transfected fibroblasts was determined using the ELISA technique (Quantikine, R & D Systems). The supernatants from irradiated or non-irradiated starting cells or EGF gene-transfected cells were removed after 24 hours and investigated for EGF production after the number of viable cells had been determined.

An irradiation was carried out at room temperature using a ^{137}Cs radiation source at a dose rate of 3 Gy/minute.

Example 2

Effect of lethal irradiation on the expression of chimeric EGF protein by gene-transfected fibroblasts

In order to achieve optimal wound-healing results, it is necessary for EGF to be available during the first days of the treatment. On the other hand, the uncontrolled in vivo growth of genetically modified cells can be prevented by lethal irradiation. The effect of lethal irradiation on the expression of EGF was therefore investigated using KMST-6 fibroblasts (clone #3, which were transfected with the plasmid pCMV-EGF-IRES-TKNeo). It was found that, although the secretion of EGF slowly declined after irradiation with 100 Gy, EGF was detectable in the supernatant in vitro over a period of at least seven days. The results are summarized in Figure 2.

Example 3

Biological activity of the chimeric EGF polypeptide

In order to demonstrate that the EGF protein which is secreted by the transfected fibroblasts also actually does exhibit biological activity, the supernatants of the media from the EGF gene-transfected clones were examined for their mitogenic activity on permanent mouse keratinocyte cell lines and on primary

human keratinocytes. Different concentrations of recombinant human EGF were used as controls. The two cell types were stimulated in a dose-dependent manner both by the recombinant protein and by the culture supernatants from clones #3 and #6. Optimal stimulation of the mouse keratinocytes was observed at a concentration of recombinant hEGF of between 2 and 20 ng/ml. This corresponds to the stimulation which can be achieved by a 1:5 dilution of the culture supernatant. The results are depicted in Figure 3 (A).

When primary human keratinocytes were used, the effective dose was marginally lower and optimum proliferation was already observed at 0.2 ng of rEGF/ml and at a 1:50 dilution of the culture supernatant from the pCMV-EGF-IRES-TKNeo/KMST6#3 clone. The results are depicted in Figure 3 (B). In both tests, it was observed that there was a tendency for cell growth to be inhibited at the higher concentrations. Culture supernatants from untransfected fibroblasts of the KMST-6 cell line did not show any stimulation of proliferation when compared with cell culture supernatants without any added growth factor. In order to investigate the therapeutic activity in wound healing in more detail, lethally irradiated EGF gene-transfected fibroblasts were cocultured in vitro with primary human keratinocytes. In the experiments, it was found that, after incubating for four days with different concentrations of irradiated, EGF-secreting fibroblasts, it was possible to obtain a dose-dependent stimulation of keratinocyte proliferation which was similar to that which was induced by recombinant growth factor. This is depicted in Figure 4, with Figure 4 (A) showing the value after four days of coculture and Figure 4 (B) showing the values after 10 days of coculture.

Example 4

Demonstration of the in vivo production of EGF by KMST-6 cells which are liposomally transfected in vitro

Experimental organization

Full-thickness skin wounds of 1.5 × 1.5 cm in size were produced on the backs of 42 nude mice. 9.4×10^6 hEGF-transfected and lethally irradiated KMST-6 cells were suspended in 2.8 ml of fibrin adhesive and transplanted onto the full-thickness skin wounds of 14 nude mice (300,000 cells/cm^2, group I).

14 full-thickness skin wounds, which were transplanted with untransfected KMST-6 cells (300,000 cells/cm^2, group II), and 14 untreated full-thickness skin wounds (group III), served as controls.

On days 1, 2, 3, 4, 5, 7 and 14, two animals from each group were in each case necropsied and 0.8 g of wound tissue was homogenized using a Triton-X/PBS buffer. The homogenates were centrifuged and the concentration of EGF in the supernatants was evaluated using an anti-human EGF ELISA.

Results

On day 1, 470 pg/ml were detectable, *in vivo*, in group I (in accordance with the invention) as compared with 18 pg/ml in group II and 1.3 pg/ml in group III. While the concentrations of EGF in group I declined on days 2-7, they were nevertheless significantly higher than in the control groups. It was not possible to detect any EGF in any of the three groups on day 14.

In summary, these results demonstrate that it was possible to successfully transplant fibroblasts which had been transfected with EGF *in vitro* in a fibrin adhesive suspension. It was possible to detect the transgenic protein *in vivo* at least up to day 7.

The results obtained in the experiment are summarized in Table I below.

Table I

Days	pCMV-EGF-IRES-TKNeo/KMST6#3 in accordance with the invention	KMST6 control (transplanted with untransfected KMST6 cells, group II)	Untreated full-thickness skin wounds (group III)
1	470	18	1.3
2	393	58	1.6
3	330	28	2.3
4	150	8	6.5
5	180	8.5	8
7	140	8.3	2.6
14	0.4	0	0

Table I shows the release of hEGF, in pg/ml, in wounds in vivo, by irradiated KMST6 fibroblasts which were transfected with a chimeric hEGF gene.

Example 5

Wound healing-promoting effect following the transplantation of KMST-6 cells, which have been transfected with EGF in vitro, in combination with human keratinocytes (mixed cell transplantation)

Experimental organization

Full-thickness skin wounds of 1.5 x 1.5 cm in size were produced on the backs of 72 nude mice. 1.0125×10^6 EGF-transfected and lethally irradiated KMST-6 cells were suspended, in combination with 2.025×10^6 human keratinocytes, in 3.6 ml of fibrin adhesive and transplanted onto the full-thickness skin wounds of 18 nude mice (ratio 1:2, 75,000 cells/cm^2, group I).

18 full-thickness skin wounds which were transplanted with EGF-transfected KMST-6 cells on their own (25,000 cells/cm, group II), 18 full-thickness skin wounds which were transplanted with human keratinocytes on their own (50,000 cells/cm^2, group III) and 18 full-thickness skin wounds which were transplanted with

untransfected KMST-6 cells in combination with human keratinocytes (ratio 1:2, 75,000 cells/cm^2, group IV) served as controls. Table II shows the division into groups.

Table II

Group I	EGF-KMST-6 + keratinocytes, n = 18
Group II	only EGF-KMST-6, n = 18
Group III	only keratinocytes, n = 18
Group IV	untransfected KMST-6 + keratinocytes, n = 18

On days 1, 3, 5, 7, 10 and 12, one animal from each group was in each case necropsied and 0.8 g of wound tissue was homogenized using a Triton-X/PBS buffer. The homogenates were centrifuged and the concentration of EGF in the supernatants was evaluated using an anti-human EGF ELISA. The other half of the biopsies was used for the histological investigations, beginning with day 5.

On post-OP days 7, 10, 12, 14, 17 and 21, biopsies from two further animals from each group were in each case examined histologically.

Results

EGF was detectable in groups I and II but not in groups III and IV.

As far as closure of the wound was concerned, the results in group I continuously showed an almost complete, pronounced epithelialization on days 7 to 12 and a complete epithelialization from day 14 onward. The group I wounds also showed the best results with regard to the quality of the reconstitution of the epidermis (cell positioning in the epithelium). By contrast, the control groups did not show pronounced epithelialization until day 14 and still did not show any high-grade epithelialization on day 21. The epithelialization is depicted in tabular form in Table III.

Table III: Tabular representation of the epithelialization

Epithelialization/differentiation

	EGF-KMST-6 + keratinocytes (group I)	EGF-KMST-6 (group II)	Keratinocytes (group III)	ut-KMST-6 + keratinocytes (group IV)
Day 5	-	-	-	-
Day 7	++	+	-	-
Day 10	++	+	+	+
Day 12	++	+	+	+
Day 14	+++	+	++	++
Day 17	+++	+++	++	++
Day 21	+++	++	++	++

- \- no epithelialization detectable
- \+ incipient epithelialization
- ++ pronounced but no complete epithelialization
- +++ complete epithelialization

Example 6

Wound healing-promoting effect in a large animal model following transplantation of KMST-6 cells which have been transfected with EGF in vitro

Experimental organization

In a large animal experiment, experiments were carried out on a total of 3 pigs (P1, P2 and P3) using a total of in each case 21 standardized burn wounds (5 cm^2 in size, grade 2a). In one pig (P1), 7 standardized burn wounds were transplanted with EGF-transfected KMST-6 cells in fibrin adhesive (therapy group). In this case, 7 untreated standardized burn wounds (control group I) and 7 standardized burn wounds which were treated with fibrin adhesive (control group III) served as controls. In two further pigs (P2 and P3), 7 standardized burn wounds were likewise in each case transplanted with EGF-transfected KMST-6 cells in

fibrin adhesive (therapy group). In each case 7 untreated standardized burn wounds (control group I) and in each case 7 standardized burn wounds which were transplanted with untransfected KMST-6 cells (control group II) served as controls. Table IV shows the division into groups.

Table IV

Therapy group	EGF-KMST-6, n = 21
Control group I	untreated burn wounds, n = 21
Control group II	untransfected KMST-6, n = 14
Control group III	only fibrin adhesive, n = 7

On days 1, 3, 5, 7, 10, 21 and 35, one wound from each group was biopsied in each case. 0.8 g of wound tissue from the biopsies up to day 10 was homogenized using a Triton-X/PBS buffer. The homogenates were centrifuged and the concentration of EGF in the supernatants was evaluated using an anti-human EGF ELISA. In addition, the biopsies were evaluated histologically.

Results

Table V shows the EGF concentration values in the groups in one wound in each case.

Table V: Tissue concentrations of EGF, in pg/ml, in the large animal experiment

	Therapy group In accordance with the invention, transplantation of EGF-transfected KMST-6 cells (P3)	**Control group I** Untreated burn wounds (P3)	**Control group II** Transplantation of untransfected KMST-6 cells (P3)	**Control group III** Treatment only with fibrin adhesive (P1)
Day 1	390	0	20	0
Day 3	200	0	20	0
Day 5	140	0	25	0
Day 7	0	0	0	0
Day 10	10	0	10	0

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Patent Claims

1) Composition for treating wounds which comprises fibroblasts, which harbor at least one foreign gene encoding a wound healing-promoting cytokine and at least one additional wound healing-promoting component.

2) Composition according to claim 1, characterized in that the wound healing-promoting component is a fibrin adhesive.

3) Composition according to claim 2, characterized in that the fibrin adhesive comprises fibrinogen and fibronectin and, where appropriate, albumin, factor XIII and plasminogen.

4) Composition according to claim 1, characterized in that the additional wound healing-promoting component is a solid component which serves as a support matrix.

5) Composition according to claim 4, characterized in that the solid component is a support matrix which is composed of a hyaluronic acid ester.

6) Composition according to one of the preceding claims, characterized in that it also comprises keratinocytes.

7) Composition according to one of the preceding claims, characterized in that the cytokine-encoding gene is selected from the group comprising the gene encoding transforming growth factor α (TGF-α), the gene encoding epidermal growth factor (EGF), the gene encoding basic fibroblast growth factor (b-FGF), the gene encoding nerve growth factor (NGF) and the gene encoding keratinocyte growth factor (KGF).

8) Composition according to one of the preceding claims, characterized in that the fibroblasts comprise at least one further foreign gene which encodes a cytokine which acts on blood cells.

9) Composition according to one of the preceding claims, characterized in that the cytokine-encoding

gene is introduced into the fibroblasts using a plasmid vector.

10) Composition according to claim 9, characterized in that the plasmid vector contains an in-frame fusion of the gene encoding the mature cytokine and a secretory signal sequence.

11) Composition according to claim 10, characterized in that the secretory signal sequence is that of the human G-CSF gene.

12) Composition according to one of the preceding claims, characterized in that the fibroblasts are autologous fibroblasts.

13) Composition according to one of claims 1 to 11, characterized in that the fibroblasts are allogenic fibroblasts.

14) Composition according to one of the preceding claims, characterized in that the fibroblasts have been irradiated.

15) Composition according to one of the preceding claims, characterized in that the fibroblasts are clonally selected fibroblasts.

16) Composition according to one of claims 13 to 15, characterized in that the allogenic fibroblasts are fibroblasts of the KMST-6 cell line.

17) Use of fibroblasts which harbor at least one foreign gene encoding a wound healing-promoting cytokine for preparing a medicament for treating wounds.

18) Use according to claim 17 characterized in that the foreign gene is selected from the genes encoding epidermal growth factor (EGF), transforming growth factor α (TGF-α), nerve growth factor [lacuna], basic fibroblast growth factor (b-FGF) and keratinocyte growth factor (KGF).

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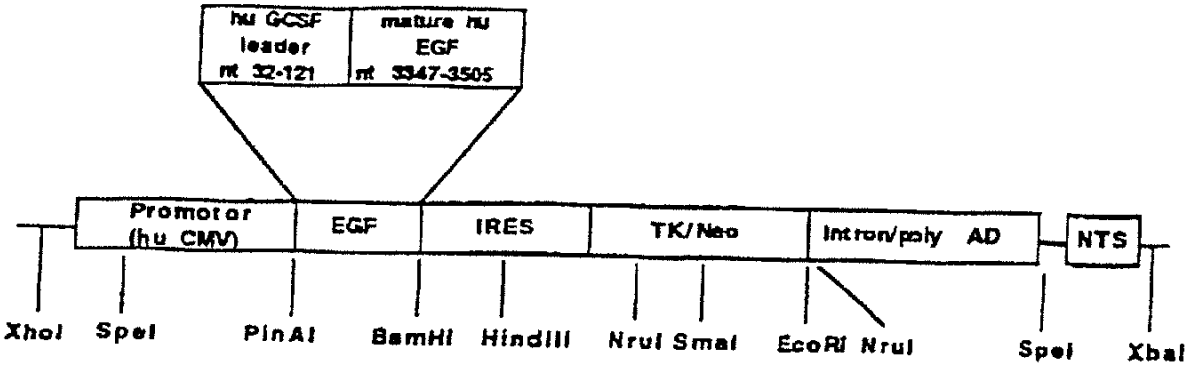
Abstract

The present invention discloses a composition for treating wounds, which composition comprises fibroblasts, which harbor at least one foreign gene encoding a wound healing-promoting cytokine, and at least one additional wound healing-promoting component.

A



B

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NheI
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CTG CAG CTG CTG CTG TGG CAC AGT GCA CTC TGG ACA GTG CAG GAA GCT agc

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp
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Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val
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Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu
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Fig. 1

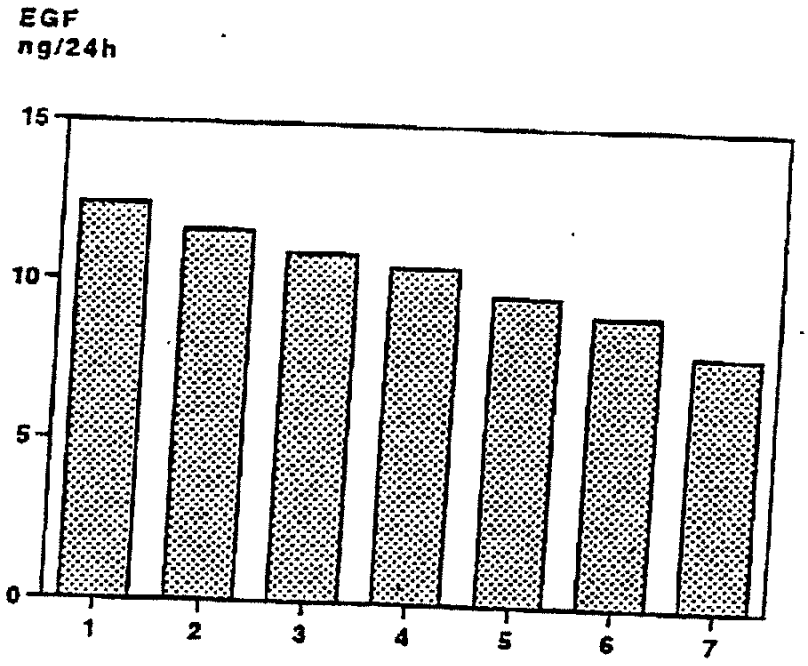
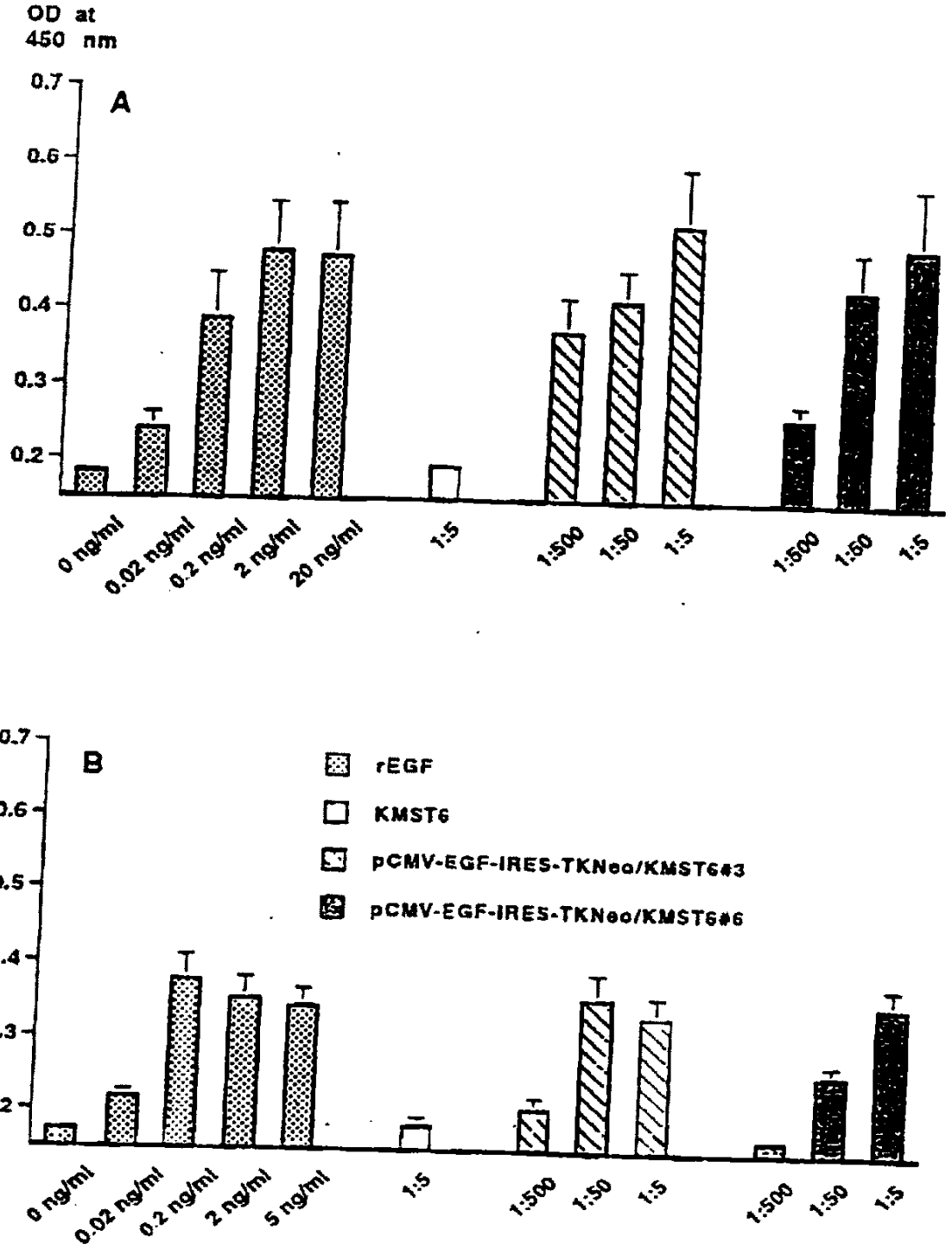


Fig. 2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1:500
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1:5
1:500
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1:5
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1:5
1:500
1:50
1:5

Fig. 3

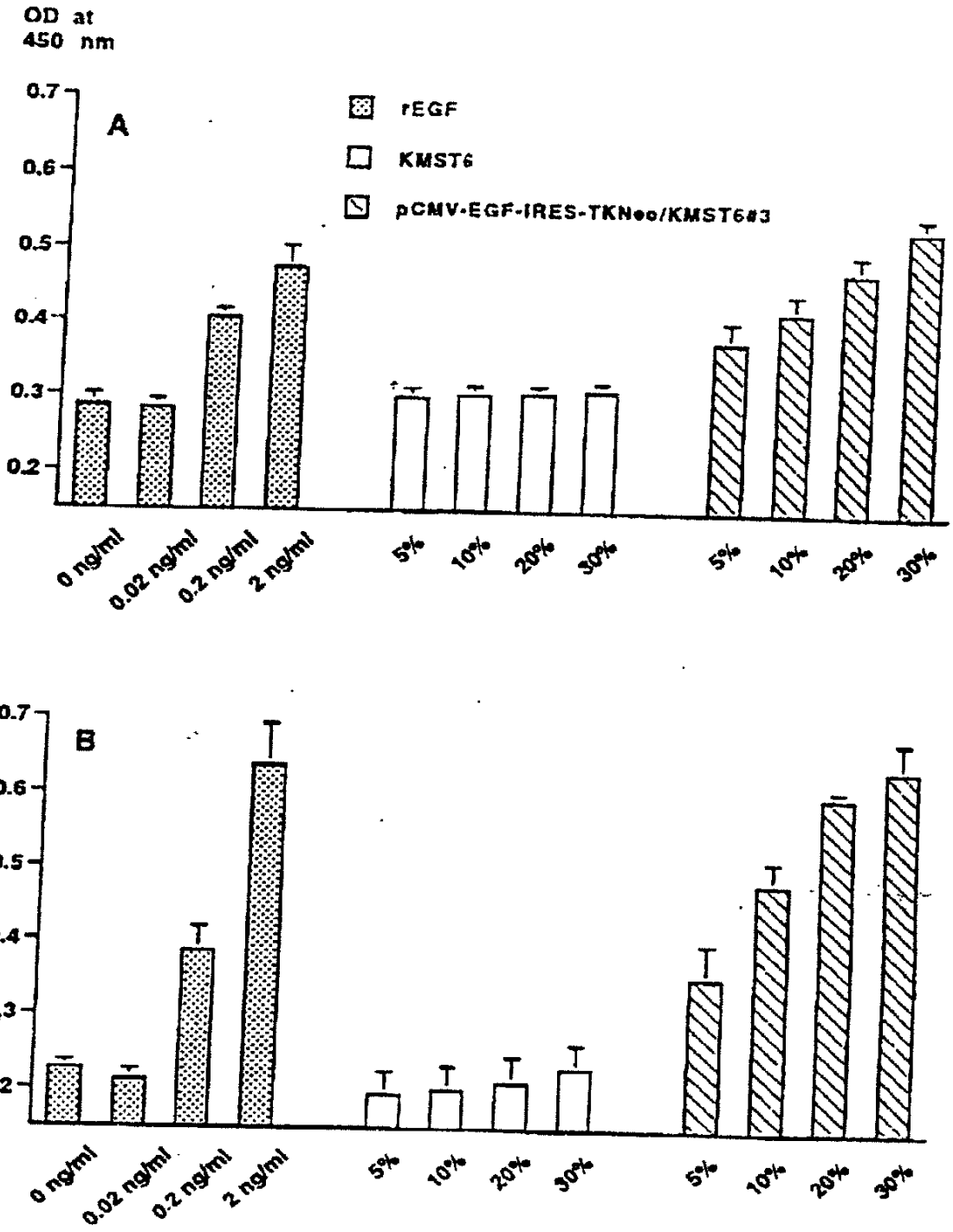
OD at
450 nm
A
rEGF
KMST6
pCMV-EGF-IRES-TKNeo/KMST6#3
0.7
0.6
0.5
0.4
0.3
0.2
0 ng/ml
0.02 ng/ml
0.2 ng/ml
2 ng/ml
5%
10%
20%
30%
5%
10%
20%
30%
B
0.7
0.6
0.5
0.4
0.3
0.2
0 ng/ml
0.02 ng/ml
0.2 ng/ml
2 ng/ml
5%
10%
20%
30%
5%
10%
20%
30%

Fig. 4